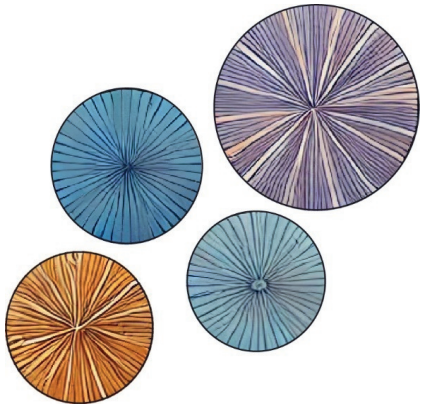
Making attachments

Summary



Summary

The musculoskeletal system is a complex system that relies on the coordinated interaction of multiple types of tissues, including tendons and ligaments, which attach to bones via specialized tissue interfaces, called entheses. The enthesis consists of a gradual transition zone that exhibits a defined extracellular matrix (ECM) composition and cell types and defined mechanical properties, which enable the transfer of mechanical forces from soft to hard tissues. Tendon and ligament ruptures are common orthopedic injuries that often lead to scar tissue formation, compromising the enthesis and impairing its function. This is primarily due to the poor self-regeneration capacity of tendons and ligaments, coupled with the complexity of the cellular and matrix composition of the soft-to-hard tissue interface. Moreover, chronic enthesitis, or inflammation of the enthesis, often results in the formation of new bone tissue at the entheseal site, which further compromises tissue function. Given the poor long-term outcomes and high injury recurrence rates associated with current treatment options, there is a pressing need to develop new strategies for tissue regeneration including drug treatment. In recent years, tissue engineering and regenerative medicine have emerged as promising approaches for enthesis healing and regeneration. Biofabrication technologies, which include both top-down and bottom-up tissue-engineering approaches, provide valuable methods for achieving this goal. Organ-on-a-chip platforms represent another promising tool for studying the key aspects of enthesis physiology or pathophysiology in the diseased state and testing drug-based therapies.

This thesis addresses the challenge of engineering a complex tissue-to-tissue junction, the enthesis, by developing novel *in vitro* models that can provide a wider range of options for investigating this crucial insertion site.

In **chapter 1**, we provide a general introduction into enthesis physiology and pathophysiology and present an overview of the current strategies used to engineer the enthesis. In **chapter 2**, we developed a microfluidic piggyback platform to enable precise control over the formation and alignment of micropatterned collagen fibers on the bottom of culture dishes. When rat tenocytes were cultured on these micropatterns, they exhibited an elongated morphology and upregulated expression of tenogenic markers at both the gene and protein levels compared to cells cultured on non-fibrillar collagen coatings or tissue culture plastic. In **chapter 3**, we increased the system complexity, by introducing three-dimensionality (3D) in an on-chip platform. The floor-ceiling chip, through topographical cues consisting of fibrillar collagen patterns on two opposing otherwise non-adhesive 2D substrates, activates

the dorsal and ventral sides of the cells in between the two substrates. Thereby, it removes the artificial polarization introduced by one-sided adhesion to a (flat) culture substrates in standard culture conditions. This strategy offers a unique opportunity to organize cells in a similar fashion to connective tissues, such as tendons or ligaments. **Chapter 4** outlines the development, fabrication and characterization of the first-ever enthesis-on-chip model. This model comprises two microfluidic chambers stacked on top of each other, with a thin, porous culture membrane in between. By inducing inflammation in the model, we were able to mimic critical features of acute and chronic enthesitis. Furthermore, we demonstrated the suitability of the model for drug screening applications by successfully mitigating the inflammatory state using the anti-inflammatory drug celecoxib. In **chapter 5**, we present a 3D spheroid model of the enthesis. Our approach involved the fusion of ligament spheroids and mesenchymal stromal cells-derived osteogenic spheroids, resulting in multicellular aggregates that expressed markers of ligament, mineralized fibrocartilage and bone. The spatial distribution of collagen-X expression within these aggregates was consistent with the hypertrophic fibrochondrocyte-containing mineralized fibrocartilage compartment of the enthesis. This approach was scalable, as demonstrated by the template-guided formation of enthesis-like tissues on the millimeter scale. Furthermore, mechanical stimulation of the enthesis spheroids by external sound waves resulted in enhanced cell proliferation and matrix deposition. In **chapter 6**, we critically evaluated the advantages and limitations of existing animal models and scaffold-based *in vitro* models for studying the enthesis. Based on this knowledge and understanding, we proposed the essential features of a potential ideal miniaturized enthesis model, outlining both the strengths and opportunities, as well as the drawbacks and challenges associated with each component.

In conclusion, this thesis provides valuable insights into the development of more advanced and representative miniaturized *in vitro* models for studying the enthesis. The new methods established in this work offer a promising roadmap for the development of future models that can better recapitulate the complex microenvironment and cellular interactions of the enthesis, including those between the cells and their ECM. This shall ultimately lead to better treatments for orthopedic injuries and diseases.